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Chemical composition and antibacterial property of *Eclipta prostrata* (L.) L.

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ABSTRACT

*Eclipta prostrata (L.) L. belonging to the Astearaceae family has been long used worldwide by various communities as a traditional medicine. We studied the antibacterial activity of different extracts from Eclipta prostrata (L.) L. against Aeromonas hydrophila, Aeromonas dhakensis, and Vibrio parahaemolyticus . The processing of this extract using repeated column and thin layer chromatographic techniques resulted in the isolation of three compounds including luteolin (**1**), kaempferol (**2**), and kaempferol-3-O-α-L-rhamnopyranoside (**3**), which were identified by NMR. Among them, kaempfreol-3-O-α-L-rhamnopyranoside was isolated for the first time and shows good antibacterial activity against Aeromonas hydrophila which is major pathogen for the aquaculture industry. The results demonstrate the beneficial effects of E. prostrata as an antimicrobial and bioactive compound for medicinal usages.*

1. INTRODUCTION

Eclipta prostrata (L.) L. has been long used by humans as a traditional medicine and hair conditioner. This plant can be easily found in various landscapes such as yards, roadsides, rice fields, gardens and neglected lands. The use of plants as traditional medicine is related to their bioactivities and their secondary metabolite. There have been several studies on the pharmaceutical application of *Eclipta prostrata* (L.) L. such as antioxidant, antiviral, antifungal, antibacterial, antihepatotoxic, anti-HIV, and larvicidal activities (Gani AMS & DN Devi Ahmad, 2015; Chung et al., 2017). Owing to the presence of various bioactive components, E. prostrata can be used as a potential herb to prevent stress-induced oxidative neuro-degeneration in Parkinson's disease (Jaisin et al., 2016). *E. prostrata also* contains various secondary metabolites, such as alkaloids, flavonoids, polyacetals, triterpenes and glycosides (Sharma et al., 2017). Therefore, this study aims to investigate the chemical composition, as well as the antibacterial activity of fractioned extracts and some isolated compounds from *E. prostrata*.

2. EXPERIMENTAL

2.1. Chemicals and reagents

NMR spectra were recorded on Bruker FTNMR spectrometer (Bruker, Karlsruhe, Germany) using TMS as an internal standard, Institute of Chemistry - Vietnam Academy of Science and Technology, Hanoi, Vietnam. TLC was performed on silica gel 60 F_{254} (0.063–0.200 mm, Merck, Germany). The zones were detected using UV at 254 or 365 nm or a solution of $FeCl_3$/EtOH or H_2SO_4/EtOH. Column chromatography was performed on silica gel (240-430 mesh, Merck.

Solvents utilized including *n*-hexane, ethyl acetate, methanol (purity ≥99.0%), and ethanol 96% were purchased from the Chemsol company (Vietnam).

2.2. Sample treatment and preparation

The aerial parts of *E. prostrata* were collected on April 2021 from Soc Trang city and authenticated by Dr. Dang Minh Quan. A voucher specimen is kept at the Department of Biology, School of Education, Can Tho University, under the number: Ecpr0421.

The sample was then washed to remove mud and dust; the rotten and damaged parts were also discarded. The raw materials were left to dry in the shade at room temperature for some days and then dried in an oven at about 50°C until well-dried.

2.3. Extraction and isolation

The well-dried plant was ground into powder (3.5 kg) which was then soaked in 96% ethanol at room temperature four times (20 L/time) and filtered. The filtrate was concentrated under reduced pressure to give brown residue as crude ethanol extract (380 g). This crude extract was then extracted on flash column chromatography successively with *n*-hexane, ethyl acetate, and methanol; then evaporated under vacuum conditions to give the residues of *n*-hexane (85 g), ethyl acetate (76 g), and methanol extract (112 g).

The ethyl acetate extract was subjected to flash column chromatography (CC) on silica gel and eluted with various proportions of *n*-hexane and ethyl acetate (from 100:0 to 0:100, v/v) to obtain eight fractions (EE1-8).

Fraction EE3 was subjected to a silica gel column and eluted with EtOAc: MeOH (from 100:1 to 10:1, v/v) to obtain seven fractions (EE3.1-7). Fraction EE3.4 was further separated on a silica gel CC and eluted with EtOAc: MeOH (from 50:1 to 0:100, v/v) to yield 12 subfractions (EE3.4.1-12). Subfraction EE3.4.8 was then subjected on silica gel CC, and compound **1** (12 mg) was obtained from subfraction EE3.4.8.4

Fraction EE6 was separated by a silica gel column and eluted with $CHCl_3$:MeOH (from 50:1 to 5:1, v/v) to yield 5 subfractions (EE6.1-5). Subfraction EE6.2 was further chromatographed on silica gel CC, eluted with $CHCl_3$: MeOH (from 10:1 to 1:1, v/v) to obtain eight subfractions (E6.2.1-8). At last, compound **2** (20 mg) was obtained from subfraction EE6.2.2.

Similarly, subfraction EE7 was subjected on silica gel CC to collect subfraction EE7.7 and then was repeatedly purified by silica gel CC to obtain compound **3** (18 mg).

2.4. Antibacterial activity

The antimicrobial activity of fractions and isolated compounds were assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and minimal inhibitory concentration (MIC) (Hossein et al., 2013). Briefly, agar plates were inoculated with bacterial strains under aseptic conditions and wells (diameter = 6 mm) were filled with 50 µL of different concentrations of samples (dissolved in DMSO) and incubated at 30°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured. Cefixim (Sigma, USA) and DMSO were employed as positive and negative controls, respectively. To determine minimal inhibitory concentration (MIC), a broth microdilution assay was employed, using Müeller-Hinton broth (MHB, Biolife, Italy) and standard bacterial inoculums (1.5×10^6 CFU/mL). Two-fold serial dilutions were prepared for final concentrations ranging from 0.3 to 5.0 mg/mL for extracts and from 8 to 128 µg/mL for pure compounds. After 24 h at 30ºC, the experiment was evaluated with no visible bacterial growth and subcultured in TSA. MIC (µg/mL) was defined as the lowest concentration of the tested substance that prevented visible bacterial growth.

The test organisms used in this study consisted of reference bacterial strains obtained from the Research Institute for Aquaculture No. 2, Ho Chi Minh City, namely *Aeromonas hydrophila*, *Aeromonas dhakensis*, and *Vibrio parahaemolyticus* which are major pathogens for the aquaculture industry.

2.5. Statistical analysis

The variation in a set of data has been estimated by performing one-way analysis of variance (ANOVA). Results were calculated from three independent experiments and are shown as mean ± SD, n=3. Results were considered as statistically significant when p value was < 0.05.

3. RESULTS AND DISCUSSION

3.1. Structure elucidation

3.1.1. Compound 1

Compound **1** was obtained as yellow needles, m.p. 303–305ºC

^{1}H-NMR (600 MHz, CD_3OD**)**, δ_H (ppm): 7.39 (1H, *dd*, 7.5 and 1.5 Hz, H-2′); 7.39 (1H, *dd*, 7.5 and 1.5 Hz, H-6′); 6.92 (1H, *d*, 7.5 Hz, H-5′); 6.55 (1H, *s*, H-3); 6.45 (1H, *d*, 1.5 Hz, H-8); 6.21 (1H, *d*, 1.5 Hz, H-6).

^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 183.9 (C-4); 166.3 (C-7); 166.0 (C-2); 163.2 (C-5); 159.4 (C-9); 151.0 (C-4′); 147.0 (C-3′); 123.7 (C-1′); 120.3 (C-6′); 116.8 (C-5′); 114.2 (C-2′); 105.3 (C-10); 103.9 (C-3); 100.1 (C-6); 95.0 (C-8).

The ^{1}H-NMR data of compound **1** revealed the presence of a pair of *meta*-coupled aromatic protons at δ_H [6.45 (1H, *d*, *J* = 1.5 Hz, H-8) and 6.21 (1H, *d*, *J* = 1.5 Hz, H-6)], an aromatic singlet (δ_H 6.55). In combination with the ^{13}C-NMR data, which showed signals for a carbonyl group at δ_C 183.9 (C-4), six oxygenated quaternary carbons (δ_C 147.0, 151.0, 159.4, 163.2, 166.0, and 166.3), two sp^2 quaternary carbons (δ_C 105.3 and 123.7), and six sp^2 tertiary carbons (δ_C 95.0, 100.1, 103.9, 114.2, 116.8, and 120.3). From these evidences and by comparison with literature data (Alwahsh et al., 2015), the molecules of compound **1** is able to identify as luteolin.

3.1.2. Compound 2

Compound 2 was obtained as light yellow powder, m.p. 275-277ºC.

^{1}H-NMR (500 MHz, CD_3OD**)**, δ_H (ppm): 8.10 (2H, *d*, 9.0 Hz, H-2′, 6′); 6.93 (2H, *d*, 9.0 Hz, H-3′, 5′); 6.41 (1H, *d*, 2.5 Hz, H-8); 6.20 (1H, *d*, 2.0 Hz, H-6).

^{13}C-NMR (125 MHz, CD_3OD), δ_C (ppm): 177.4 (C-4); 165.6 (C-7); 162.5 (C-5); 160.6 (C-4′); 158.3 (C-9); 148.1 (C-2); 137.1(C-3); 130.7 (C2′, 6′); 123.8 (C-1′); 116.3 (C3′, 5′); 104.6 (C-10); 99.3 (C-6); 94.5 (C-8).

The ^{1}H-NMR spectrum of compound **2** showed four signals of six aromatic protons, in which there were two couples of chemical shift equivalent protons at δ_H [8.10 (2H, *d*, *J* = 9.0 Hz) and 6.93 (2H, *d*, *J* = 9.0 Hz)]; two *meta*-coupling signals at δ_H [6.41 (1H, *d*, *J* = 2.5 Hz) and 6.20 (1H, *d*, *J* = 2.0 Hz)]. ^{13}C-NMR and DEPT spectra also exhibited signals of total 15 carbons of a flavone backbone. These carbons consisted of two signals of two couples of chemical shift equivalent carbons at δ_C 116.3 (2C) and 130.7 (2C), related to two couples of chemical shift equivalent protons in its ^{1}H-NMR spectrum. It proved that the flavones containing four hydroxyl groups had a symmetric aromatic ring. Moreover, the 1D-NMR spectral data of compound **2** were similar to those of kaempferol reported in the literature (Nenadis et al., 2004). From these results, compound **2** was determined as 3,5,7,4′-tetrahydroxyflavone or kaempferol.

1.1.3 Compound 3

Compound (**3**) was obtained as yellow amorphous powder, m.p. 177–179ºC.

^{1}H-NMR (500 MHz, DMSO-d_6**)**, δ_H (ppm): 7.74 (2H, *dd*, 7.0 and 2.0 Hz, H-2′, 6′); 6.90 (2H, *dd*, 7.0 and 2.0 Hz, H-3′, 5′); 6.39 (1H, *d*, 2.0 Hz, H-8); 6.19 (1H, *d*, 2.0 Hz, H-6); 5.29 (1H, *d*, 1.5 Hz, H-1′′); 0.79 (1H, *d*, 6.0 Hz, H-6′′).

^{13}C-NMR (125 MHz, DMSO-d_6), δ_C (ppm): 177.6 (C-4); 161.2 (C-7); 160.0 (C-5); 157.1 (C-2); 156.5 (C-9); 134.1 (C-3); 130.5 (C 2′, 6′); 120.5 (C1′); 115.4 (C-3′, 5′); 103.9 (C-10); 101.8 (C-1′′); 98.8 (C-6); 93.8 (C-8); 71.1 (C-2′); 70.6 (C-3′); 70.3 (C-4′); 70.5 (C-5′); 17.4 (C-6′′).

The ^{1}H-NMR spectrum of compound **3**, showed two *meta*-coupled proton signals at δ_H 6.19 (1H, *d*, *J* = 2.0 Hz) and δ_H 6.39 (1H, *d*, *J* = 2.0 Hz), corresponding to H-6 and H-8 of the A-ring protons, respectively. A typical AA′BB′ system at 7.74 ppm (2H, *dd*, *J* = 7.0 and 2.0 Hz, H-2′, H-6′) and δ_H 6.90 (2H, *dd*, *J* = 7.0 and 2.0 Hz, H-3′, H-5′) confirmed the 1,4-disubstituted B-ring. These data indicated that the aglycon of compound **3** is kaempferol. The anomeric proton signal at δ_H 5.29 (*d*, *J* = 1.5 Hz, H-1′′), in addition to the one doublet at δ_H 0.79 (*J* = 6.0 Hz, H-6′′) observed in the ^{1}H-NMR spectrum of compound **3** established the presence of one rhamnose unit. The downfield shifts of H-6 and H-8 of the aglycone compared to those of H-6 and H-8 of kaempferol and the HMBC showed that rhamnose moiety was attached to the C-3 position. Therefore, compound **3** was established as kaempferol-3-O-α-L- rhamnopyranoside and in good agreement with the reported literature (Ghaly et al. 2014).

Three compounds **1-3** were isolated and identified from the aerial parts of *E.prostrata* , including luteolin (**1**), kaemferol (**2**), and kaempferol-3-O-*α*-L-rhamnopyranoside (**3**) by analysis of their NMR spectra and comparison with literature data (Figure **1**). The compound (**3**) is isolated first time from this species.

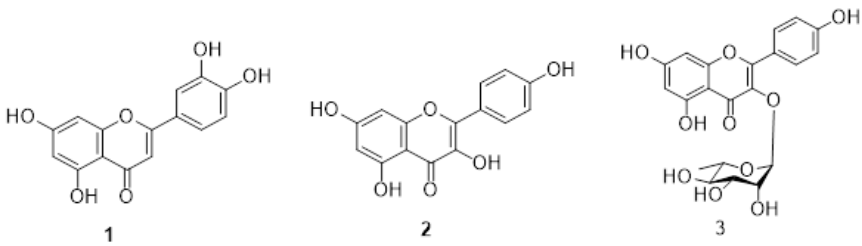

Figure 1. Structures of compounds 1 - 3

3.2. In vitro antibacterial results

Ethyl acetate extract and kaempferol-3-O-*α*-L-rhamnopyranoside showed high antibacterial effects against all bacterial strains including *Aeromonas dhakensis*, *Aeromonas hydrophila*, and *Vibrio parahaemolyticus* with MIC values against bacterial strains ranging between 39 and 625 µg/mL for extracts and from 8 to 32 µg/mL for isolated compounds. Kaempferol-3-O-*α*-L-rhamnopyranoside showed good antibacterial activities against *Aeromonas hydrophila* tested bacterial at 128 µg/mL with zone of inhibition 25.33 ± 0.35 mm and MIC = 8.0 µg/mL (Table **1**).

Previously, ethyl acetate extract of *E. prostrata* was reported to exhibit antimicrobial activity against many strains of microorganisms with MICs of 1.56–25.00 mg/mL (Borkataky et al., 2013). These investigated microorganisms, i.e., *M. catarrhalis, S. pyogenes* and *C. diphtheriae* are human pathogenic bacteria causing various kinds of diseases. Another report showed that the three new thiophene derivatives, ecliprostins A-C (1-3), have been isolated from the aerial parts of the medicinal plant *Eclipta prostrata* and all three compounds showed antibacterial activity against *Staphylococcus aureus* (Yu et al., 2020). Flavonoids are well known as antibacterial agents against a wide range of pathogenic microorganisms. In this study, the antibacterial activities of the crude extract and fractions as well as pure compounds isolated from *E. prostrata* were carried out with the organisms, namely *Aeromonas hydrophila, Aeromonas dhakensis,* and *Vibrio parahaemolyticus* which are major pathogens for the aqualculture industry. The present results further confirm the activity of the extracts and constituents isolated from *E. prostrata* against tested bacteria and justify the potential use of this medicinal plant in folk medicine, as well as expand our knowledge on the bacterial activity of this species. Some of the compounds isolated are candidates for further work to evaluate their therapeutic potentials, especially in aquaculture.

Table 1. Zone of inhibition and MIC of extracts and isolated compounds

Extracts and isolated compounds		Diameter of growth inhibition zones at different concentrations					MIC
Extracts (mg/mL)		0.3125	0.625	1.250	2.500	5.000	(µg/mL)
crude extract	*A. hydrophila*	13.43^{E}±0.21	14.37^{C}±0.21	15.40^{C}±0.20	15.93^{B}±0.15	16.43^{A}±0.23	312
	A. dhakensis	15.17^{E}±0.15	15.83^{D}±0.06	16.53^{C}±0.25	17.53^{B}±0.15	18.13^{A}±0.25	78
	V. parahaemolyticus	13.37^{E}±0.15	14.47^{C}±0.15	15.53^{C}±0.38	16.77^{B}±0.06	17.50^{A}±0.30	312
n-hexane extract	*A. hydrophila*	9.13^{E} ±0.15	10.30^{D}±0.10	11.43^{C}±0.25	12.60^{B} ±0.27	13.63^{A} ±0.21	312
	A. dhakensis	12.23^{E} ±0.21	14.33^{D}±0.15	16.20^{C}±0.20	17.33^{B} ±0.15	19.23^{A} ±0.21	625
	V. parahaemolyticus	11.23^{E} ±0.21	13.20^{D}±0.20	13.83^{C}±0.06	14.53^{B} ±0.31	16.20^{A} ±0.17	312
ethyl acetate extract	*A. hydrophila*	14.37^{E} ±0.21	16.57^{D}±0.21	19.53^{C}±0.15	22.13^{B} ±0.15	22.80^{A} ±0.10	39
	A. dhakensis	16.33^{E} ±0.58	19.37^{D}±0.15	22.50^{C}±0.20	23.33^{B} ±0.21	24.63^{A} ±0.15	39
	V. parahaemolyticus	16.43^{E} ±0.23	17.53^{D}±0.23	19.53^{C}±0.15	20.30^{B} ±0.20	21.23^{A} ±0.21	78
Isolated compounds (µg/mL)		8	16	32	64	128	
kaemferol	*A. hydrophila*	14.05^{E} ±0.21	15.50^{D}±0.10	16.64^{C}±0.20	17.07^{B} ±0.21	17.30^{A} ±0.10	16
	A. dhakensis	12.12^{E} ±0.31	14.13^{D}±0.21	16.44^{C}±0.21	17.50^{B} ±0.27	18.67^{A} ±0.21	32
	V. parahaemolyticus	12.40^{E} ±0.30	14.23^{D}±0.15	15.60^{C}±0.17	16.45^{B} ±0.20	17.47^{A}±0.41	32
kaempferol-3-O-*α*-L-rhamnopyranoside	*A. hydrophila*	14.70^{E}±0.17	15.73^{D}±0.21	17.39^{C}±0.31	24.60^{B}±0.27	25.33^{A}±0.35	8.0
	A. dhakensis	12.60^{E}±0,10	14.87^{D}±0,06	15.33^{C}±0,15	16.83^{B}±0,06	17.57^{A}±0,15	16
	V. parahaemolyticus	11.57^{E}±0,15	11.93^{D}±0,06	12.47^{C}±0,25	14.30^{B}±0,27	15.53^{A}±0,21	32
cefixim	*A. hydrophila*	22.33^{C}±0.58	23.00^{C}±1.00	26.67^{B}±2.31	30.67^{A}±1.53	31.67^{A}±0.58	8.0
	A. dhakensis	-	-	-	9.67^{B}±0.58	14.33^{A}±0.58	32
	V. parahaemolyticus	-	-	-	10.00^{B}±0.00	12.67^{A}±0.58	32

4. CONCLUSION

The results of this study revealed that from the aerial parts of *Eclipta prostrata* (L.) L., grown in Soc Trang city, we have isolated and identified three compounds: luteolin (**1**), kaemferol (**2**), and kaempferol-3-O-*α*-L-rhamnopyranoside (**3**) in which compound **(3)** was isolated for the first time from this species. The structures of these compounds have been elucidated by NMR spectroscopy and comparedwith the literature data. Furthermore, the antibacterial potentials of the fractioned extracts and isolated compounds were also evaluated through the agar well diffusion method and broth microdilution assay. The results indicate that ethyl acetate extracts have the good antibacterial propterties and the compound kaempferol-3-O-*α*-L-rhamnopyranoside exhibited the good antibacterial activity against *Aeromonas hydrophila*.

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